

The Effects of Aggregations on Water Loss in *Collisella digitalis*

by

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Abstract. Water loss rates were compared between isolated and aggregated *Collisella digitalis* of the same microhabitat. The solute concentration of the extra-corporeal water (ECW) was used as an indicator of water loss. Limpets within conspecific aggregations tended to have lower ECW solute concentrations than isolated limpets. Isolated limpets tended to orient with their head down on vertical surfaces, although this tendency was absent in the aggregations.

INTRODUCTION

HIGH-INTERTIDAL MARINE organisms are exposed to air with each tidal cycle. Prolonged calm periods and neap tides can increase the length of exposure, isolating some splash-zone animals in an essentially terrestrial environment for several days (WOLCOTT, 1973). While exposed, marine animals are vulnerable to evaporative water loss due to wind and solar radiation. Many animals subject to these conditions have physiological adaptations, such as high desiccation tolerance, that allow persistence in the intertidal. In addition, these animals may have behaviors that affect the physical parameters governing water loss, thereby reducing desiccation stress.

Collisella digitalis (Rathke, 1833), a high intertidal snail, demonstrates many behaviors that impede water loss when it is exposed to air. This acmaeid limpet occurs from the Aleutian Islands, Alaska, to the southern tip of Baja California (MORRIS *et al.*, 1980), predominately on vertical rock surfaces (HAVEN, 1971; COLLINS, 1976). Unlike the homing limpet *C. scabra*, *C. digitalis* does not fit exactly to the substrate. This leaves a gap between the shell and substrate through which water is lost. The mucous sheet formed by *C. digitalis*, and other limpets, significantly reduces this water loss, acting as a physical barrier to exchange (WOLCOTT, 1973). *Collisella digitalis* often aggregates in crevices and depressions during low tide (FRANK, 1965). Presumably the topographic relief provided by these sites reduces water loss rates, although this has yet to be demonstrated. *Collisella digitalis* also forms conspecific ag-

gregations on smooth rock surfaces which lack such topographic relief. HAVEN (1971) suggested that these aggregations reduce water loss from the animals but provided no supporting data.

In this study, I have examined the effects of clumping on water loss in *Collisella digitalis* by comparing solute concentrations of extra-corporeal water (ECW) between clumped and isolated limpets. The extra-corporeal water, held between the foot and shell, is the source of evaporative water loss from limpets (SEGAL, 1956). As freshwater is evaporated from the ECW, its solute concentration increases. Although ECW solute concentration is an indirect assessment of water loss, it is valuable in that data can be collected in the field.

MATERIALS AND METHODS

Specimens of *Collisella digitalis* were sampled during May, 1983, at three sites along the northern California coast: (a) Blind Beach, Sonoma County; (b) the Bodega Marine Laboratory, Sonoma County; and (c) Albion, Mendocino County. Most of the samples were taken from the smooth chert at Blind Beach. Individuals were sampled *in situ* at varying temperatures, wind conditions, and times of day. All samples were taken from smooth surfaces, well away from any crevices or depressions.

Freehand sketches of aggregations and nearby isolates served as permanent references of the position of each individual sampled. Usually eight limpets were sampled: four from within the aggregation, and four isolates from the surrounding area. For comparison, an isolate needed to be at least 4 cm from its nearest neighbor, but not more than 30 cm from the aggregation. This assured that the eight limpets, hereafter treated as a class, were from a similar microhabitat.

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Table 1

Solute concentration data for aggregated (Cl) and isolated (Is) individuals of *Collisella digitalis*.

Class ¹	Number of individuals		Average osmotic pressure (mOsm/kg)		Osmotic pressure difference	P ²	Relative change in concentration with time ³		
	Cl	Is	Cl	Is			Cl	Is	X ⁴
1	4	3	1362	1516	156	>0.20	245	399	0.385
2	5	3	1500	1723	223	<0.01	385	606	0.368
3	3	3	1420	1730	310	0.18	303	613	0.506
4	3	3	1180	1370	190	0.14	63	253	0.751
5	5	6	1041	1158	117	<0.01	—	—	—
6	6	5	1041	1118	77	0.02	—	—	—
7	3	3	986	966	-20	>0.20	—	—	—
8	3	3	1160	1176	16	>0.20	43	59	0.271
9	4	4	1187	1330	143	<0.01	70	213	0.671
10	4	3	1120	1243	123	<0.01	3	126	0.976
11	3	3	1126	1280	154	0.18	9	163	0.945
12	4	4	1215	1287	72	0.15	98	170	0.423
13	4	4	1135	1175	40	>0.20	18	58	0.690
14	5	4	1142	1205	63	0.17	25	88	0.716
15	4	4	1200	1375	175	>0.20	83	258	0.678
16	4	4	1230	1762	532	<0.01	113	645	0.825
17	4	4	1153	1460	307	0.09	36	343	0.895
18	4	4	1233	1355	222	<0.01	116	238	0.933
19	4	4	1206	1355	149	<0.01	89	238	0.626
20	4	4	1106	1147	41	<0.01	—	—	—
21	4	4	1092	1147	55	<0.01	—	—	—

¹ Clumped and isolated individuals occupying the same microhabitat.

² P-value for Student's *t*-test. Comparisons were between clumped and isolates' average osmotic pressure.

³ Derived by subtraction of estimated starting concentration (1117 ± 39 mOsm/kg) from average osmotic pressure.

⁴ Solute concentration ratio for Cl:Is as calculated using Equation 1 (see text).

Limpets were removed from the substrate with a narrow spatula. A 16-μL sample of ECW was quickly removed from the posterior foot-shell margin with a microcapillary tube. The full tube was then capped with Seal Ease clay to inhibit further evaporation from the sample, and numbered to correspond with the sketch. The length of each individual was measured using calipers, after which the limpet was dipped in seawater and returned to the rock. The vapor pressure of each sample was measured in the laboratory using a Wescor Inc. 5130C Vapor Pressure Osmometer, which gave the ECW vapor pressure in milli-osmoles per kilogram (mOsm/kg). Vapor pressure of a sample is directly proportional to solute concentration.

All limpets on one rock (n = 211) were censused to determine their orientation. The direction of the anterior end of each limpet was scored according to an eight point

compass. An individual with its head straight up was scored as 1, straight down, as 5. One-way Chi-square tests were used for each group (clumped and isolates) to determine whether the orientation of individuals was random.

RESULTS

In all but one of the 21 classes sampled, the average ECW solute concentration of clumped limpets was lower than that of nearby isolates (Table 1). The range of differences between average solute concentrations was -20 to +532 mOsm/kg. In this pairwise comparison within classes, the size of the individuals was not considered. However, the volume of ECW determines the rate at which the concentration changes. To assess the role of size, an analysis of covariance was run using size as the covariate. Pooling the data in this way demonstrated that the ECW solute concentration of clumped limpets was lower than that of isolates ($F_{1,155} = 19.85$, $P < 0.01$). In addition, limpets in aggregations were arbitrarily ranked according to the degree to which they were surrounded. A completely surrounded individual was ranked 4, a limpet with no close neighbors was ranked 0. An analysis of covariance using the ranked positions as a covariate to solute concentration demonstrated that limpets with few close neighbors had higher solute concentrations than those that were completely surrounded. Consequently, those limpets at the periphery of the aggregation (usually rank 2) tended to have higher ECW solute concentrations than individuals in the interior ($F_{1,71} = 4.38$, $P < 0.05$) (Figure 1).

Due to the paired nature of the sampling procedure, relative rates of water loss between the two groups can be estimated. Fourteen limpets, eight of which were aggregating, were sampled to estimate the ECW concentration at the beginning of the dichotomy. The ECW solute concentration for these limpets was 1117 ± 39 mOsm/kg. The concentration change over the exposure period can thus be calculated by subtraction, and the values used to compare average concentration changes of the clumped and isolated limpets given by Equation 1 as

$$\frac{I - C}{I} = X$$

where C and I are the change in ECW solute concentration for the clumped and isolated limpets respectively. The average value for X was 0.67 ± 0.22 for all the classes used in the comparison (five were omitted because their final ECW solute concentrations were lower than the assumed starting value). Assuming that all animals in each class had been exposed for approximately the same period of time, the proportion X represents a difference in water loss rates. As such, the clumped limpets lost water 33% slower than isolates (Table 1).

Analysis of orientation on the substrate (Figure 2) revealed that neither the clumped nor the isolated limpets were randomly situated ($\chi^2 = 20.33$, $P < 0.05$ for clumped;

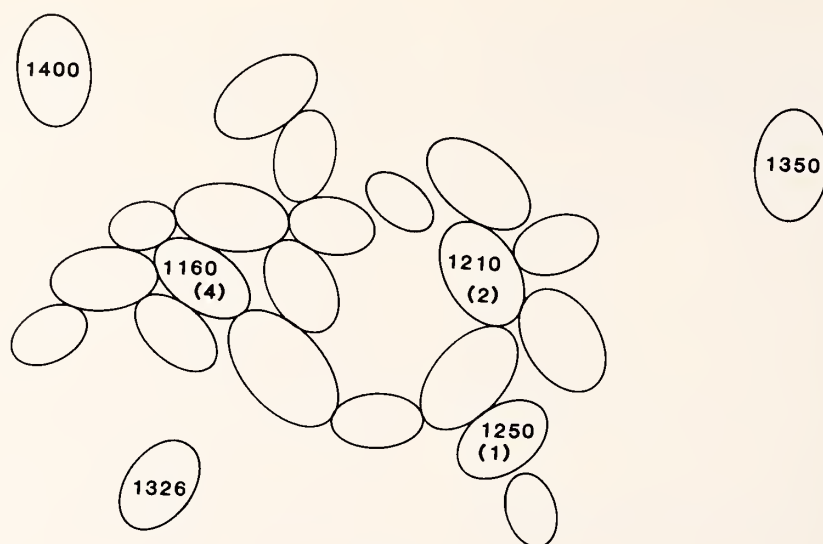


Figure 1

Diagram of a typical aggregation of *Collisella digitalis* and surrounding isolates. The ECW solute concentration (mOsm/kg) and rank (in parentheses) of some of the limpets from Class 19 are given. The positions of the isolated limpets have been changed to reduce the figure size.

$\chi^2 = 143.58$, $P < 0.01$ for isolates). The trend for the isolates was to orient with the head down. The trend for the clumped limpets was less obvious, but the most common orientation was with the head to the right.

DISCUSSION

Clumping has been shown to reduce water loss rates in both marine and terrestrial invertebrates. WARBURG (1968) and ALLEE (1926) showed that terrestrial isopods in ag-

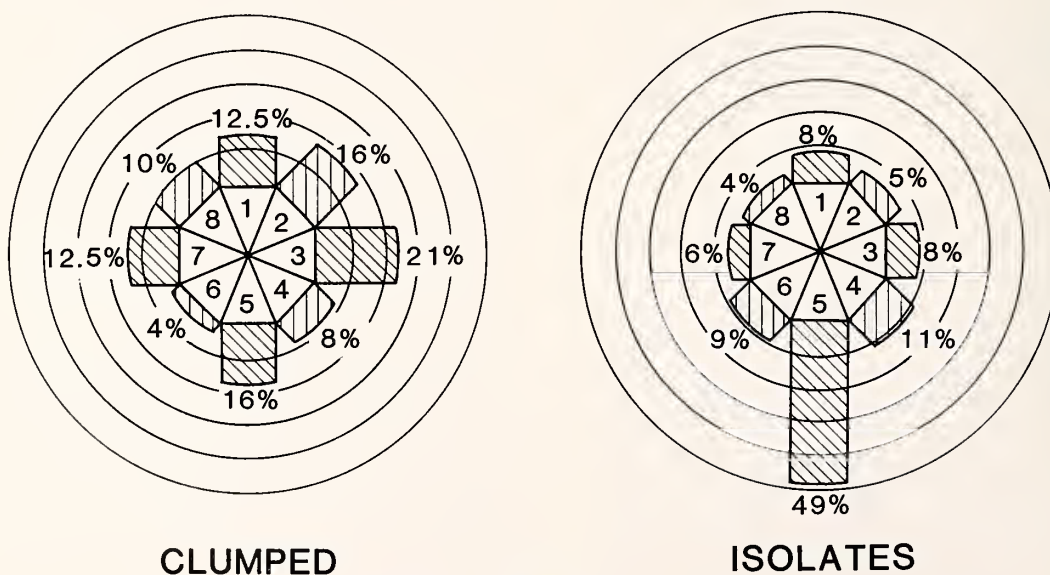


Figure 2

The orientation of *Collisella digitalis*, clumped or isolated, on vertical surfaces. The percentage figures are the relative frequency of each position with respect to vertical. The striped bar extends in the same direction as the anterior of the limpet. ($n = 94$ for aggregated, clumped, limpets; $n = 117$ for isolates.)

gregations lose water at one-half the rate of isolates. In addition, clumped hermit crabs survived longer under desiccating conditions than isolated crabs (SYNDER-CONN, 1981). The data presented in this paper clearly show a similar trend for *Collisella digitalis*: clumped limpets lost less water during the exposure period than isolated limpets. The reduction of water loss is most likely attributable to reduced wind velocities within the aggregation. Perimeter animals exposed to the wind would slow wind speed due to friction (drag). This notion is supported by the finding that perimeter animals (rank 2) have higher ECW solute concentrations than limpets within the aggregation. However, I did not resolve which solutes resulted in the ECW concentration changes. Thus, it is possible that the observed concentration differences are due to the addition of other solutes such as urine to the extra-corporeal water. I have conducted preliminary studies on the biophysics of this system. The data gathered so far support the hypothesis that wind is the primary effector of water loss, and that wind velocities are lower within the aggregation.

There is indirect evidence that further supports the hypothesis that isolated limpets are under more extreme desiccation stress than clumped limpets. WOLCOTT (1973) reported that *Collisella digitalis* produces a mucous sheet that impedes water loss. I observed, but did not sample, several isolated limpets with mucous sheets, and I saw no clumped limpets with the barriers. Those isolates with the barriers had little extra-corporeal water, suggesting that the mucous sheets form as the ECW dries, preventing further water loss from the body tissues.

The orientation of isolated limpets may also indicate the severity of their condition. ABBOTT (1956) proposed that the head-down orientation observed in *Lottia gigantea* would ensure that the head and ctenidium would be the last to dry as the ECW volume decreased due to evaporative losses. The present work supports this hypothesis in that isolated limpets tended to orient with their head down on vertical surfaces (see also MILLER, 1968). In addition, some isolated limpets that had been subjected to acute dehydration, where almost all of the ECW was removed, were dried and discolored except in the head region. Interestingly, the tendency to orient head down is absent in clumped limpets, suggesting that the rigors of desiccation are reduced within an aggregation. Nevertheless, clumped limpets are subjected to evaporative water loss; however, position within the aggregation may be a more important factor governing their orientation.

The behavior of *Collisella digitalis* is consistent with the finding that the aggregations represent areas of reduced water loss. The crevices and depressions where the limpets often occur stay moist throughout the tidal cycle, limiting water loss because of moist air, cool temperature, and perhaps reduced wind speeds. ALLEE (1926) reported that isopods form aggregations when ordinary shelter is unavailable, thus satisfying the same tactile reaction. Simi-

larly, *C. digitalis* forms conspecific aggregations on smooth rock surfaces where no topographic shelter exists. This suggests that the aggregations are analogous to crevices with respect to shelter, and perhaps desiccation resistance.

The combination of aggregation, exploitation of crevices, and formation of mucous sheets, probably accounts for the persistence of *Collisella digitalis* in the high intertidal. The inability to fit exactly to the substrate renders the limpets vulnerable to environmental fluctuations. By forming aggregations, *C. digitalis* can effectively reduce the rates of evaporative water loss, thus reducing the degree of physiological stress normally imposed by its environment.

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LITERATURE CITED

- ABBOTT, D. P. 1956. Water circulation in the mantle cavity of the owl limpet, *Lottia gigantea* Gray. *Nautilus* 69(3):79-87.
- ALLEE, W. C. 1926. Studies in animal aggregation: causes and effects of bunching in land isopods. *J. Exp. Zool.* 45:255.
- COLLINS, L. S. 1976. Abundance, substrate angle and desiccation resistance in two sympatric species of limpets, *Collisella digitalis* and *Collisella scabra*. *Veliger* 19:199-203.
- FRANK, P. W. 1965. The biodemography of an intertidal snail population. *Ecology* 46:831-844.
- HAVEN, S. B. 1971. Niche differences in the intertidal limpets *Acmaea scabra* and *Acmaea digitalis* (Gastropoda) in central California. *Veliger* 13(3):231-248.
- MILLER, A. C. 1968. Orientation and movement of the limpet *Acmaea digitalis* on vertical rock surfaces. *Veliger* 11(suppl.): 30-44.
- MORRIS, R. H., D. P. ABBOTT & E. C. HADERLIE. 1980. Intertidal invertebrates of California. Stanford University Press: Stanford, Calif. 241 pp.
- RATHKE. 1833. *Acmaea*. In: Eschscholtz's "Zoologischer Atlas," Heft 5, pp. 16-21, 23-24.
- SEGAL, E. 1956. Adaptive differences in water holding capacity in an intertidal gastropod. *Ecology* 37:174.
- SYNDER-CONN, E. K. 1981. The adaptive significance of clustering in the hermit crab *Clibanarius digueti*. *Mar. Behav. Physiol.* 8:43.
- WARBURG, M. R. 1968. Behavioral adaptations of terrestrial isopods. *Amer. Zool.* 8:545.
- WOLCOTT, T. G. 1973. Physiological ecology and intertidal zonation in the limpets (*Acmaea*): a critical look at limiting factors. *Biol. Bull.* 145:389-422.